REMARKS

Claims 1-103, 105, and 109 have been previously cancelled without prejudice or disclaimer. Claims 104 and 110 have been amended to recite a "method for determining cancer prognosis or prediction for a human subject" in the preamble. Support for the amendment can be found throughout the specification, for example, at paragraphs [0018], [0081], [0088], [0089], and [0096], and in the Examples. Thus, the amendment is fully supported by the specification and no new mater has been added. The amendments to the claims were made solely in the interest of expediting prosecution, and are not to be construed as acquiescence to any objection or rejection of any claim.

Upon entry of these amendments, claims 104, 106-108, and 110-113 are under consideration.

Claim Rejections under 35 U.S.C. § 103

Claims 104, 106, 108 and 110-113

Claims 104, 106, 108, and 110-113 are rejected under 35 U.S.C. §103 as allegedly being unpatentable over Dai et al. (US 2003/0224374) in view of Duvick (US Patent No. 7,026,123), Clement et al. (J. Biol. Chem. 276:16919-16930, 2001), Lipson et al. (PNAS 86:9774-9777, 1989), Chang et al. (J. Neuroscience Methods 94:177-185, 2000), and Matsubara et al. (Endocrinology 138:5075-5078, 1997). The Office appears to allege that Dai et al. taught all of the elements of the claims, except that Dai et al. "did not teach targeting intronic sequences; i.e. Dai did not teach that the probes of the microarray were designed to hybridize to *intronic RNA* or nucleic acids produced therefrom." Office Action at p. 6 (emphasis original). Additionally, the Office acknowledges that "Dai did not teach quantitative PCR using a primer complementary to an intronic RNA sequence." *Id.* Thus, the Office combines Duvick, which allegedly taught methods for examining the effects of transforming clones of nucleic acid sequences into host cells, where each clone comprised a candidate sequence and a "U-tag," a short, random nucleotide sequence that may "be designed into an intron sequence that occurs anywhere within a transcript." *Id.* The Office alleges that Duvick suggested "introns could be detected, and that the level of the detected intron correlated with transcription rate (i.e. expression)" because

Duvick stated that "the spliced-out intron RNA would be detected at a level proportional to the transcription rate. Recent data from mammalian cells indicates that intron RNAs, after splicing, persist in the cell with reasonable half-lives, contrary to what was previously thought." *Id.* at p. 6-7.

Additionally, the Office combines Clement et al. because Clement et al. allegedly taught that introns from the Pem gene are more stable (have longer half-lives) than the $IVSI_{C\beta1}$ intron, which was detectable by the "relatively insensitive Northern blot procedure." *Id.* at p. 7. The Office further combines Lipson et al., Matsubara et al., and Chang et al. because they allegedly "all used intron-specific primers or probes to quantify gene expression." *Id.* at p. 8. Thus, the Office alleges that it would have been *prima facie* obvious to one of ordinary skill in the art to modify the method suggested by Dai et al. by measuring expression based on the detection of intron sequences as suggested by Duvick. The Office further alleges that based on the disclosures of Clement et al., Lipson et al., Matsubara et al. and Chang et al., one of skill in the art would have had a reasonable expectation of success that the levels of detected intron sequence would be proportional to the transcription rate, which the Office alleges is a measure of gene expression, and a reasonable expectation of success in quantitatively detecting the sequences given the quantitative densitometry taught by Lipson and the quantitative PCR taught by Matsubara et al.

In response to Applicants' prior response, the Office contends that "transcription rate" can be considered a measure of gene expression, just as "steady state level of mRNA" or "level of protein" can be considered measures of gene expression. *Id.* at p. 12. The Office alleges without any support that "[t]hese different measures of gene expression would not all produce the same 'number', but each would be a reflection of 'gene expression.'" *Id.* The Office also alleges without any support that "introns and exons (mRNA) would not need to be degraded at the same rate for each to correlate with gene expression." *Id.* at p. 13. Without providing any evidence, the Office states that:

> The point is that for a particular gene, the steady-state level of mRNA and the steady state level of a particular intron of the gene, though different, would each have been expected to correlate with the expression of the gene: the more highly expressed the gene, the more mRNA and the more intronic RNA from that gene would be present.

Id. The Office continues to state that:

> For any given intron sequence, a steady-state level would exist in the cell, and that this steady-state level would correlate with the expression of that particular gene, as suggested by Duvick. Therefore, because Duvick suggested that introns would be detected at a level proportional to the transcription rate, and because the other prior art cited in the rejection clearly showed that such intron sequences were detectable, it would have been obvious to target intron sequences for measuring gene expression in the method of Dai.

Id. at p. 14. Applicants respectfully disagree.

"[W]hen an examiner relies on a scientific theory, evidentiary support for the existence and meaning of that theory ***must be provided***." *In re Grose*, 592 F.2d 1161, 201 U.S.P.Q. 57 (CCPA 1979); MPEP §2144.02 (emphasis added). In making the rejection, the Office relies on scientific theories without providing any evidence to support them. Specifically, the Office broadly alleges that the transcription rate is a measure of gene expression and that this was suggested by Duvick. As shown below, at the time of the earliest filing date of the instant application, the transcription rate was not in general considered representative of a measure of gene expression. In fact, as discussed in more detail below, Duvick suggested that the transcription rate as measured by intronic RNA levels does NOT correlate with mRNA levels. Furthermore, the Office alleges that the steady-state level of an mRNA and the steady-state level of an intron of that gene would be expected to correlate with gene expression. In other words, the Office alleges that "the more highly expressed the gene, the more mRNA and the more intronic RNA from that gene would be present." Office Action at p. 13. Evidence available before the earliest filing date of the instant application indicates that this trend was not generally accepted. Thus, one skilled in the art would not have been motivated to use an "intronic RNA sequence of a target gene . . . to determine the expression level of the target gene . . . and determining cancer prognosis or prediction . . ." as recited in the claims. In contrast, Applicants unexpectedly found that intronic RNA can serve as a prognostic or predictive molecular marker.

First, the Office appears to rely heavily on Duvick's statement that "intron RNA would be detected at a level proportional to the transcription rate" and erroneously makes the assumption that the transcription rate also correlates with mRNA levels or gene expression. While intron RNA levels may in some circumstances correlate with the transcription rate, it was not generally accepted that the intron RNA levels (or transcription rate) correlate with mRNA

levels or gene expression. Indeed, nowhere does Duvick teach or suggest that intron levels correlate with mRNA levels or gene expression. In fact, Duvick suggests the contrary:

> An advantage of intron localization of tags would be that ***variations in mRNA stability of coding regions would not be a factor in accumulation of tag RNA***.

Col. 6, lines 11-13 (emphasis added). Duvick recognizes that the level of intron RNAs detected may be independent of mRNA levels because it does not take into account post-transcriptional effects on mRNA, such as mRNA stabilization and mRNA destabilization, which may affect mRNA levels but not transcriptional activity. Thus, based on the teachings of Duvick, one skilled in the art would not have reasonably expected a correlation between intron RNA levels (or transcription rate) and mRNA levels and therefore, would not have been motivated to use an "intronic RNA sequence of a target gene . . . to determine the expression level of the target gene . . . and determining cancer prognosis or prediction . . ." as recited in the claims.

Evidence in the art also suggests that transcription rate was not generally accepted as correlating with mRNA levels. For example, Fan et al. compared the change in mRNA steady-state levels (x_i) and change in transcription levels (y_i) for a number of genes following several conditions of stress (UV, heat shock, and treatment with prostaglandin A_2 (PG)). Fan et al., "Global Analysis of Stress-Regulated mRNA Turnover by Using cDNA Arrays," *PNAS* 99:10611-10616 (Aug. 6, 2002), at p. 10612, col. 2, first full paragraph. Fan et al. classified the genes into the following five groups based on the observed changes:

> Group I: "comprises genes whose expression do not change with stress" but included in this group were "***also a small set of genes exhibiting no change in steady-state levels, whose transcription either increased or decreased***."
>
> Group II: "comprises genes whose total mRNA levels increase after stress and their transcription rates also increase."
>
> Group III: "encompasses genes whose total mRNA levels decrease following stress and their transcription rates are similarly reduced."
>
> Group IV: "genes showed ***sizeable increases in total RNA abundance, while their transcription rates remained unchanged***."
>
> Group V: "genes show ***marked decreases in steady-state levels, while their transcription rates are typically unchanged***."

Id. (emphasis added).

Thus, Fan et al. identified a number of genes whose transcription rates did not correlate with steady-state levels of mRNA and that did not follow "the more highly expressed the gene, the more mRNA and the more intronic RNA from that gene would be present" trend asserted by the Office. For example, post-transcriptional *mRNA stabilization increased* mRNA abundance of Group IV genes while transcription remained largely unchanged. *Id.* Similarly, post-transcriptional *mRNA destabilization decreased* mRNA steady-state levels of Group V genes while transcription remained largely unchanged. *Id.* Fan et al. found that mRNA stabilization and destabilization played a role in more than half of stress-regulated genes analyzed, and emphasized the "prominent role" of post-transcriptional effects on gene expression:

> Use of this methodology provided systematic confirmation that changes in transcription influenced the altered expression of many stress-regulated genes, about 47% in the current analysis. However, it also revealed that ***mRNA stabilization and destabilization significantly influenced the expression of approximately 53% of stress-regulated genes, underscoring the prominent role of mRNA turnover as a major contributor in the implementation of stress-altered gene expression patterns.***

Id. at p. 10611, col. 1, second full paragraph.

Similarly, Carey et al. found that "the regulation of H-FABP [heart fatty acid-binding protein] expression may or may not be at the level of transcription depending on the stimulus." Carey et al., "Transcriptional Regulation of Muscle Fatty Acid-Binding Protein," *Biochem. J.* 298:613-617 (1994), at Abstract. When Carey et al. stimulated fatty acid utilization in rats by either streptozotocin (STZ)-induced diabetes or fasting, Carey et al. found a correlation between H-FABP protein levels and mRNA levels in both sets of animals. *Id.* at p. 616, col. 2, fourth full paragraph ("Table 1 shows that STZ-induced diabetes and fasting cause an increase in H-FABP and mRNA levels"). However, Carey et al. observed different H-FABP transcription levels in animals with STZ-induced diabetes and those that underwent fasting, despite the fact that both groups of animals displayed similar increases in H-FABP mRNA levels. *Id.* at Table 1; p. 616, col. 2, last paragraph. Thus, Carey et al. concluded that "in the case of STZ-induced diabetes, ***an increase in transcription rate cannot account for the increase in H-FABP mRNA***. These results imply that the expression of H-FABP in skeletal muscle can be regulated at several levels including transcriptional activation and probably mRNA stability." *Id.* at p. 617, last paragraph (emphasis added).

Even Matsubara et al., which the Office cited, reported similar findings. Matsubara et al. measured transcriptional activity of the growth hormone (GH) gene by measuring nuclear GH pre-mRNA (intronic RNA) levels [Matsubara et al. at p. 5075, col. 1, second full paragraph ("kinetics of transcription activity of GH gene parallels the kinetics of nuclear GH pre-mRNA levels")] and compared it to mature mRNA levels. *Id.* at p. 5077, col. 1, first and second paragraphs. Matsubara et al. found that levels of nuclear GH pre-mRNA (hence, the transcription rate) did not correlate with levels of mature mRNA at any given time. Specifically, Matsubara et al. found that dexamethasone (Dex) and triiodo-L-thyronine (T3) "significantly increased GH pre-mRNA levels" after 6 hrs of incubation, while there were no significant changes in GH mRNA levels. *Id.* at Abstract. After 24 hrs of incubation with Dex and T3, significant increases in GH mRNA levels were detected but GH pre-mRNA levels did not differ between treated and non-treated cells. *Id.* Thus, Matsubara et al. concluded that:

> The cellular levels of mature GH mRNA are affected not only by the transcription activity, but also by the stability and degradation of the molecules.

Id. at p. 5077, col. 2, first full paragraph.

Accordingly, one skilled in the art would not have had a reasonable expectation that intron RNA levels or transcription rate would correlate with gene expression. Therefore, the mere suggestion by Duvick that detection of intron RNA levels would be proportional to the transcription rate would not have motivated one skilled in the art to modify the method of Dai et al. to use an "intronic RNA sequence of a target gene . . . to determine the expression level of the target gene . . . and determining cancer prognosis or prediction . . ." as recited in the claims.

Nothing in Clement et al., Lipson et al., Chang et al., and Matsubara et al. make up for the deficiencies of Dai et al. and Duvick. Matsubara et al. has already been discussed above. Clement et al. only teaches that the half-lives of *Pem* introns may be longer than that previously believed and teach nothing about the correlation between intron levels and mRNA levels. Moreover, Clement et al. studied the introns from the *Pem* gene, which the Office asserted "appear to be typical introns." Office Action at p. 7. While Clement et al. began their study of *Pem* introns because they initially appeared to be "typical" of introns, Clement et al. found that they were, in fact, "unique" and possibly different from other introns. "Surprisingly," the *Pem* introns were found in the cytoplasm, unlike other introns such as the TCR-β spliced intron

$IVS1_{c\beta1}$. Clement et al. at Abstract and p. 16924. Thus, the study by Clement et al. may be unique to the *Pem* introns and may not apply generally to all introns.

Lipson et al. tested for intron-containing mRNA precurors (hnRNA) of the thymidine kinase (TK) gene in the active S-phase and quiescent G_o phase of human diploid fibroblast (WI-38) cells using RT-PCR. Lipson et al. at Abstract. Lipson et al. explained that detection of hnRNAs reflects transcriptional activity of the TK gene. Lipson et al. at p. 9774, col. 2, first full paragraph; p. 9776, col. 2, last paragraph to p. 9777, first paragraph. However, as discussed above, detection of the intron in hnRNA does not generally provide correlative information regarding mRNA levels or gene expression, which can be affected by post-transcriptional and/or post-translational activity. Although Lipson et al. also measured mRNA levels, Lipson et al. appears to have used mRNA simply as a control in the RT-PCR method because hnRNAs are more difficult to detect. For example, Lipton et al. found that the limits of detection were 2000 cells in S phase for TK hnRNA and 20 cells for the mature mRNA. *Id.* at p. 9775, col. 2, first paragraph. Lipson et al. determined that TK hnRNA is maximally expressed early in the S phase of the cell cycle after quiescent human fibroblasts are stimulated to proliferate, and that at this point, the TK hnRNA to TK mRNA ratio was 1:155. *Id.* at Abstract. However, this ratio does not indicate whether the intron-containing hnRNA correlates with mRNA levels or gene expression. In fact, as evidenced above, one skilled in the art would not have had a reasonable expectation that intron levels (or transcription rates) would correlate with mRNA levels or gene expression.

The Office quoted Chang et al., stating that "estimates of transcription rates for the TH gene based on relative levels of intron 2 sequences are well founded. The logical extension and underlying rationale of the present study is the application of intron-specific in situ hybridization analysis for studies on TH gene expression . . . ." Office Action at p. 8. However, while Chang et al. shows that intron RNA levels correlate with transcription rate, nothing in Chang et al. teaches or suggests that the intron RNA levels (or transcription rate) correlate with mRNA levels or gene expression. As discussed above, one skilled in the art would not have had a reasonable expectation that intron levels (or transcription rates) would correlate with mRNA levels or gene expression.

In summary, the mere suggestion by Duvick that detection of intron RNA levels would be proportional to the transcription rate would not have provided one skilled in the art with a

reasonable expectation that intron RNA levels or transcription rate would correlate with gene expression. Thus, one skilled in the art would not have been motivated to modify the method of Dai et al. to use an "intronic RNA sequence of a target gene . . . to determine the expression level of the target gene . . . and determining cancer prognosis or prediction . . ." as recited in the claims. In contrast, Applicants unexpectedly found that intronic RNA can serve as a prognostic or predictive molecular marker. Accordingly, Applicants believe that the claims are non-obvious and are patentable. Withdrawal of the rejection is respectfully requested.

Claim 107

Claim 107 is rejected under 35 U.S.C. §103 as allegedly being unpatentable over Dai et al. in view of Duvick, Clement et al., Lipson et al., Chang et al., and Matsubara et al., as applied to claims 104, 106, 108 and 110-113 above, and further in view of Danenberg et al. (US 2002/0009795). The Office acknowledges that Dai et al., Duvick, Clement et al., Lipson et al., and Matsubara et al. do not teach or suggest a FFPE sample as recited in claim 107. Office Action at p. 10. However, the Office alleges that Danenberg et al. teaches a method that "provides simple, efficient and reproducible methods for the isolation of RNA, DNA or proteins from tissue that has been embedded in paraffin." *Id.* Thus, the Office argues that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to apply the method suggested by the combined teachings of Dai et al., Duvick, Clement et al., Lipson et al., Chang et al., and Matsubara et al. to samples of FFPE tissue using the RNA isolation method taught by Danenberg et al.. *Id.* at p. 11. Applicants respectfully disagree.

The deficiencies of Dai et al., Duvick, Clement et al., Lipson et al., Chang et al., and Matsubara et al. were discussed above. Nothing in Danenberg et al. makes up for these deficiencies. Danenberg et al. teach isolation of RNA from formalin-fixed paraffin embedded tissue but teach nothing about the correlation of intronic RNA to mRNA levels or gene expression. Accordingly, Applicants believe that claim 107 is non-obvious and patentable. Withdrawal of the rejection is respectfully requested.

CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone James Keddie at (650) 833-7723.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number GHDX-007.

Respectfully submitted,
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